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Domain Swapping in Crystals of *E. coli* trp Repressor Grown from Aqueous Isopropanol.

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Beamline(s):X12B, X12C, X25

Introduction: Our current structural view of *E. coli* trp repressor (trpR) as a compact alpha-helical homodimer is based largely on crystallographic and NMR studies performed in high salt buffers (1,2). We are investigating the structure of trpR crystals grown in 30% aq. isopropanol. The crystals diffract relatively poorly, therefore a synchrotron radiation source is required to obtain diffraction data beyond 3.5 Angstrom.

Methods and Materials: Hexagonal bipyramid crystals of trpR were grown by vapor diffusion over a reservoir of 30% (v/v) isopropanol, 100 mM HEPES pH 7.5, and 100 mM NaCl, then transported to the NSLS. Crystals were transferred for 1-2 minutes to a cryoprotectant solution of the recipe above plus 25% ethylene glycol, then flash-cooled to 100 deg. K on a nitrogen gas stream. A 5-Bromotryptophan derivative crystal was prepared by soaking it in reservoir solution plus 10mM 5-Bromo(DL)tryptophan for two days prior to flash-cooling. For all diffraction measurements, the wavelength of incident radiation was set to approximately 1.0 Angstrom.

Results: The crystals were determined to have space group symmetry P6(1)22, $a=85.3$ Angstrom, $c=113.5$ Angstrom, and contain one 107 residue trpR subunit per a.u. with 75% solvent content. Native diffraction data were collected from a single crystal at X12C to 3.1 Angstrom, and from a second crystal at X25 to 2.45 Angstrom. The structure was solved by molecular replacement and is now refined against the X25 data to $R\text{-free} = 0.295$, $R = 0.279$, using TLS parameters to model disorder (3). Bromotryptophan derivative data collected at X12B to 3.3 Angstrom were used to calculate a model-phased difference Fourier map which yielded a 13.7 sigma positive peak at the expected Br position in the L-tryptophan binding pocket. This result verifies that the molecular replacement solution is correct and it demonstrates that the L-tryptophan binding pocket remains intact in this crystal form.

Conclusions: In crystals grown from 30% aq. isopropanol, individual trpR subunits have a dramatically extended conformation, and yet they assemble into compactly folded units that strongly resemble the conventional trpR dimer fold. The extended conformation results from the coalescence of helices C, D and E and their intervening turns into a single long helix. In an extreme example of domain swapping, each extended trpR subunit contributes N-terminal helices (A-C) to one cfu and C-terminal helices (E-F) to a neighboring cfu. In solution, addition of alcohol to globular proteins is known to increase helical content and promote unfolding. This may be the first instance where these effects are observed for a protein in a crystalline state. Similar extended subunit structures may contribute to trpR aggregation phenomena in low salt (4).

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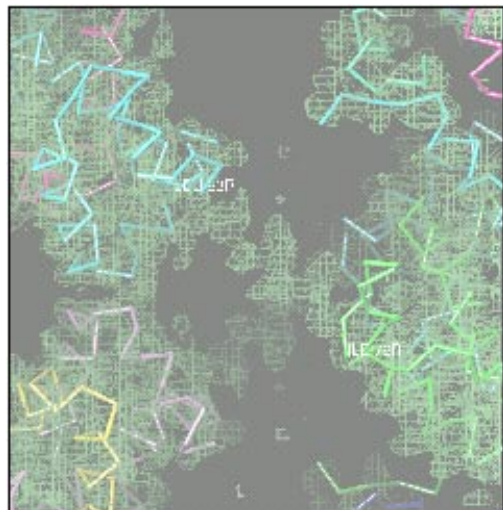


Figure 1. Electron density map based on X25 diffraction data reveals a dramatic conformational change involving domain swapping. Molecular replacement model shown.